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HAEMATOLOGY AND SERUM BIOCHEMISTRY OF COCKERELS GIVEN *Vernonia amygdalina* LEAF EXTRACT IN A SUB-TROPICAL ENVIRONMENT

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Poultry is particularly sensitive to environmental challenges, especially heat stress. Climatic condition such as heat stress has a negative effect on poultry production thereby altering their behaviour and physiological homeostasis. The means of acquiring synthetic pharmaceuticals are not easily obtainable to small scale farmers, therefore there is an urgent need for cheap, unconventional source of antioxidant such as *Vernonia amygdalina* (VALE) to alleviate the adverse effect of heat stress. A total of 45 cockerels were randomly distributed into 5 treatment groups, replicated 3 times with 3 birds per replicate under elevated summer temperature to investigate the effect of *Vernonia amygdalina* leaves extract supplementation via drinking water on their haematology and serum characteristics which lasted for 8 weeks. Parameters evaluated under haematological profile were: Packed cell volume, white blood cell, haemoglobin concentration, red blood cell, platelet, mean corpuscular haemoglobin, mean cell volume, mean corpuscular haemoglobin concentration while under serum biochemistry; Triglceride, total cholesterol, HDL, LDL, sodium, calcium, alkaline amino phosphase, alkaline amino transferase, aspartate amino transferase, total antioxidant potential were evaluated. The results of proximate analysis shows a chemical composition of 1295.19KJ/mole Calorific value, 53.03% CHO, 23.68% CP, 7.32% CF, Crude lipids 0.3%, 8.90% ash. The result of phytochemical screening revealed saponin (+++), tannin (+++), phenol (+++), flavonoid (++), steroid (++), glycosides (++) and anthraquinone (++). The presence of these essentials nutrients and minerals implies *Vernonia amygdalina* could be utilized as a source of feed supplement to improve blood profile and serum biochemistry of heat-stressed poultry. Results obtained for serum biochemistry shows a significant different in triglyceride for birds in Treatment 2 in which the value recorded was lower. Aspartate amino transferase also shows a significant difference in Treatment 4 which is the highest value that receives 60mls of the extract although not significantly different across the treatment group. Result obtained for Haematological profile shows that there is no significant different in all treatment groups but a higher concentration was observed in treatment 5 which received 90mls of the extract compared with the control group.
In conclusion, VALE at 90mls can be used as a natural source of antioxidant. Further studies can be conducted using higher concentration of VALE.

Keywords: *Vernonia amygdalina*, heat stress, cockerel, leave extract, haematology, serum

Production of domesticated birds for consumption is a potential way of solving the problem of low animal protein intake in the growing human population. This claim is supported by the reports of some researchers such as Al-Fataftah and Adu-Dieyeh (2007) which reported a 33% increase in poultry production in Jordan between 1985 and 2005, Kale, (2011) who also reported a 7.7% increase in production in Nigeria between 2006 and 2010. Also, It has been reported that the total world poultry meat production increased by 39% between the year 2000 and 2010 (59 and 82 million tonnes eviscerated weight, respectively (Terry, 2010). However, heat stress in broiler chickens is of global concern to poultry

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farmers in both temperate and tropical regions of the world (Balnave, 2004). The impact of heat stress on birds depends on the environmental temperature in combination with the level of RH and the duration of exposure (Widowski, 2010). A rise in CBT by 4°C leads to death among birds (DEFRA, 2005). Heat stress negatively also affects feed intake, growth rate, meat quality, egg production, immune function and so can lead to higher mortality (Mashaly *et al*., 2004. Collectively, these negative effects could reduce the sustainability of commercial broiler chicken production and have severe economic implications for the broiler industry (St-Pierre *et al*., 2003). Steps to promote sustainable poultry production in tropical regions could include genetic improvement of the indigenous breeds which are already adapted to the prevailing environmental conditions, or providing genetically-improved broiler genotypes with structures inside the poultry house to alleviate conditions of heat stress (Sonaiya,1993). Unfortunately, installation of such features in developing countries may not be feasible because of the high capital cost and lack of constant power supply (Gous and Morris, 2005). Also, continuous use of synthetic sources of pharmaceutical such Ammonium Chloride, Sodium bicarbonate in animal production could store up in animal tissue and have transference effect on humans consuming the resulting animal products. Interest has developed recently in the collection and extended use of medicinal plant extract such as *Vernonia amygdalina* known to have strong natural antioxidant and other properties for an extended use of production purpose (Griggs and Jacob, 2005).

MATERIALS AND METHODS

Experiment was carried out at Kwara State University Teaching and Research Farm Malete, Kwara State, in a southern guinea savannah ecological zone of Nigeria. With average day time temperature of 23-36°C and relative humidity of 60 -80%. Forty five cockerels of 21 weeks old were purchased for the experiment. The weights of the birds ranged from 3.0-4.0kg and they are acclimatized for 2weeks in a 4-tyre cage for physiological adjustment to the environment. Grower mash was fed to the birds and clean fresh water is provided *ad libitum.* The rectal temperature was monitored throughout the experiment. The leaves of *Vernionia amygdalina* were harvested from University Teaching and Research Farm, rinsed with distilled water and air dried at room temperature (23°C) for ten days before blending into powder with electronic blender for proximate and phytochemical analysis in a reputable laboratory. The powdered sample weighing was kept in an airtight container before taking it for analysis. The proximate analysis parameters were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). All analysis was carried out in duplicate. Phytochemical screening to test for the presence of Saponin, Tannins, Anthraquinones, Steroids, Glycosides (Keller-Killiani Test), Flavonoids for Alkaloids was performed using standard procedures (Ayoola *et al.,* 2008).

Extraction procedure

Distilled water was boiled at 99°C. The 500mls of the boiled distilled water was then added to the 50g Vernonia powder so that 1g of *Vernonia amygdalina* powder received 10mls of boiled distilled water. The mixture was first stirred with a rod and was later placed on a magnetic stirrer for 4-6 hours, after which the mixture was sieved. The mixture obtained was filtered into a conical flask. The extract was then stored in a refrigerator before use and the process was repeated every week throughout the experimental period.

Experimental design

The birds were divided into five treatments and each treatment has three replicates with three birds per replicate. T1 served as the control, which received ordinary water and T2 contained ascorbic acid (Vitamin C 0.4mg/litre). Treatments 3, 4 and 5 contained concentrated *Vernonia amygdalina* leaf extract at 30, 60 and 90mls/litre respectively. The design of the experiment was complete randomized design

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and all data was analyzed using one way analysis of variance.

Blood collection

At the end of the experiment, blood samples were randomly collected from the wing vein of four randomly selected birds in each treatment for heamatological and serum biochemical studies. Each blood sample was drawn by means of sterile disposable (21-guage) syringe and needle into sample bottles containing dipotassium salts of ethylene diamine tetra-acetic acid (EDTA) for heamatological studies while samples for biochemical indices were collected into anticoagulant-free bottles and allowed to clot. Sera were obtained after the blood sample has been allowed to stand for two hours at room temperature and centrifuged for ten minutes at 2000 rpm to separate the serum from the blood sample.

Statistical analysis

All data obtained from this investigation were subjected to analysis of variance of completely randomized design using the SAS (1999) package and the means were separated using Duncan multiple range test of the same software. The model used was;

$Yij = \mu + Ti + eij$

Where: Yij = Individual observation, μ = overall mean, Ti = treatments effect and eij = residual error.

RESULTS AND DISCUSSION

TABLE 1: Proximate composition of *Vernonia amygdalina* harvested from Kwara State University Teaching and Research Farm, Malete, Kwara State.

Table 1: Proximate composition of *Vernonia amygdalina*

PARAMETERS	VALUES (%)
Crude Fibre	7.33
Ash	8.90
Moisture	6.68
Carbohydrate	53.03
Calorific value	1295.19
Crude Lipid	0.37
Crude Protein	23.68

Table 1 shows the result of nutrient composition of *Vernonia amygdalina* leaves obtained from Teaching and Research Farm, Kwara State University. The result revealed that *Vernonia amygdalina* leaves contain perceptible amount of crude protein (23.68%), crude fibre (7.32%), ash (8.90%), crude lipids (0.37%), moisture (6.68%), CHO (53.03%) and Calorific value (1259.19). The CP value obtained in this study is higher than the CP reported by Okoli *et al.,* 2003 reported as 17%. Due to its high content of crude protein, it was found to be a good source of plant protein which could be used to enhance poultry performance, for animal growth. The crude fibre of 7.33% was observed in this study. Fibre cleanses the digestive tract by removing potential carcinogens from the body and inhibits the absorption of excess cholesterol (Mensah *et al.,* 2008). It also adds bulk to the diet and prevents the intake of excess starchy food (Mensah *et al.,* 2008) and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus (Henry, 2004). Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Rao and Newmark, 1998). The ash content of *Vernonia amygdalina* (8.90%) is a reflection of the mineral contents preserved in the plants leaf. The ash content of *Vernonia amygdalina* leaves observed in this studyis lower compared with the ones reported for *Vernonia colorate* (15.86%) and *Moringa oleifera* (15.09%) (Antia *et al.,* 2006) but higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). *Vernonia amygdalina* had a crude lipid value of 0.37%, thus contributing to the energy value of the leaves. Dietary fat increases the palatability of food by absorbing and retaining flavours (Antia *et al.,* 2006). The moisture content value of *Vernonia amygdalina* leaves was relatively low. The low moisture content is desirable as it would hinder the growth of spoilage microorganisms and enhance shelf life (Ruberto and Baratta, 2000). The presence of these nutrients implies that *Vernonia amygdalina* can be utilized as a valuable nutrient, healthy for poultry, and could be

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used in preventing diseases that are related to malnutrition.

Table 2: Phytochemical screening of *Vernonia amygdalina* leaf extract

PHYTOCHEMICALS	*VERNONIA AMYGDALINA* (%)
Saponin	+++
Flavonoid	++
Tannins	+++
Alkaloids	++
Phenol	+++
Steroid	++
Glycoside	++
Anthraquinone	++

Where; +++ = high level, ++ = moderate level.

The results of the phytochemical screening of the ethanolic leaf extract of *Vernonia amygdalina* showed the presence of various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, phenol, steroid, glycosides and anthraquinone (Table 2). These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. The qualitative phytochemical screening of *Vernonia amygdalina* was in agreement with the works of Foong and Hamid, (2012) and Vijayameena *et al* (2013). It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Ugwu *et al.*, 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al.*, 2013; Pourmorad *et al.*, 2006). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al.*, 1995). Plants that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001). Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity Also, several alkaloid-containing medicinal plants are reported to have been used by the early man as pain relievers. (Gurib-Fakin, 2005). Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.*, 1995). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Okwu and Emineke, 2006). Glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al.*, 1995). These finding suggest that this plant is a potential source of natural antioxidants that could serve great importance as therapeutic, anti-inflammatory, anti-analgesic and anti-hyperlipidemic agent

The result obtained for the haematological analysis of cockerels randomly assigned to Treatments 1 to 5 is shown in Table 3. The result showed that all parameters assessed were not significantly different across the treatment except those of platelets, WBC and Lymphocytes. Birds given 30mls of *Vernonia amygdalina* leaf extract (VALE) had the highest numerical values in PCV, Hb, MCV, MCH, while the lowest values were recorded in birds given T2 given 0.4g of Vit C. Packed cell volume measures the percentage of red blood cell to the total blood volume. Osho *et al.* (2014) demonstrated that oral administration of VALE on broiler chickens did not have a significant effect on Packed cell volume (PCV), Haemoglobin (Hb), and Red blood cells (RBC) in treated birds. Packed cell volume measures the percentage of red blood cell to the total blood volume. Acute inflammation from most pathogenic microorganism as well as other causes result in haemolysis which is manifested in lower Hb level and PCV (*Kumarnsit et al.*, 2006) as observed in birds given Vit C.

The WBC values were significantly influenced across the treatment, with the birds given Vit C at 0.4ppm showing the highest recorded value while the lowest

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Table 3: Haematological indices of cockerels given varying concentrations of VALE

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
PCV (%)	32.33	30.67	38.00	37.33	33.00	3.70
Hb (g/dl)	10.77	10.22	12.67	12.44	11.00	1.16
WBC ($x10^3$)	245.77^{ab}	263.47^{a}	248.00^{ab}	245.70^{ab}	237.87^{b}	33.49
RBC (%)	2.02	2.67	2.06	1.09	2.91	0.44
PLT($X10^9$)	5.33^{ab}	8.00^{a}	4.33^{ab}	4.67^{ab}	6.33^{a}	1.51
LYMPH	1.33^{b}	4.00^{a}	0.00^{b}	0.00^{b}	0.67^{b}	0.60
MCH(pg)	56.64	39.84	62.60	56.13	40.52	7.26
MCV(fl)	16.97	11.95	18.77	16.83	12.16	2.18
MCHC	0.69	0.89	1.02	0.63	0.97	0.15

Table 4: Serum biochemical response of cockerels given varying concentrations of VALE

Parameters	**T1 (0 VALE)**	**T2 (VitC0.4ppm**	**T3 (30mls VALE)**	**T4 (60mls VALE)**	**T5 (90mls VALE)**	**SEM**
Trig(mg/dl)	27.91^{a}	13.87^{b}	20.66^{ab}	20.19^{ab}	22.56^{ab}	3.28
T chol(mg/dl)	277.87	88.54	241.54	179.50	142.75	64.96
HDL-C(mg/dl)	2.72	1.86	5.12	3.41	1.86	1.22
LDL-C(mg/dl)	248.62	74.30	219.69	158.63	119.82	63.65
Na ppm	105.1	200.00	120.20	32.40	27.20	77.82
AST	76.53	73.80	78.33	78.87	74.00	0/22
Ca ppm	1.35	1.24	1.22	1.05	1.13	0.11
T.P (mg/dl)	9.20	9.06	8.78	9.00	8.89	0.23
ALP (U/L)	6.90	4.14	4.09	5.06	1.59	2.01
ALT (U/L)	24.47	24.47	25.00	24.27	24.67	0.23
AST (U/L)	76.53	73.80	78.33	78.87	74.00	0.22
TAP (mg/dl)	62.50	52.02	45.17	45.88	44.42	1.19

Keywords: PCV% (Packed cell volume), HB (g/dl) (Haemoglobin concentration), WBC×10^3 (White blood cell), RBC% (Red blood cell), PLT×10^9(Platelet), T.P (mg/dl) Total Protein MCH (pg) (Mean corpuscular haemoglobin), MCV(fl) (Mean cell volume), MCHC (g/dl) (Mean corpuscular haemoglobin concentration).

value (T5) was observed in cockerels given 90mlsVALE. An increase in the value of WBC is an indication that infection is present. The lowest WBC obtained in T5 could be attributed to the anti-bacterial and anti-parasitic properties of VALE against an incidence of infection. The values obtained for RBC ranged from 1.86-2.91x10, with the highest recorded in T5. Higher RBC value is an indication of a nutritious and healthy diet composition. The presence of VALE at 90mls/L could have enhanced erythropoiesis with no destructive effect on the red blood cells. A lymphocyte is one of the subtypes of white blood cell in a vertebrate's immune system. An increase in lymphocyte concentration is usually a sign of a viral infection . A high lymphocyte count with a

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low neutrophil count might be caused by lymphoma. Platelets of birds given T2 (8.00x 10^9/ mm^3)) and T5 (6.33 x 10^9/mm^3) were recorded to be significantly raised, while those of T3 and T4 were statistically similar compared with the control value (5.33 x 10^9/ mm^3) The main function of platelets is to contribute to platelet plug (primary hemostasis): which is involved in the process of stopping bleeding at the site of interrupted endothelium (Yip *et al*., 2005). The higher value of these haematological indices in the treatment administered with the extract can be due to their inability to cause hemolysis which could result from the anti-inflammatory properties inherent in *Vernonia amygdalina*. Cole (1986) reported that caged birds have a PCV of 35%-55% and a PCV less than 35% indicates anemia while the one greater than 55% suggest dehydration. The PCV values were obtain within the normal range. This signifies that there were no symptoms of physiological anemia or dehydration in the experimental birds.

Table 4 shows the serum biochemistry of cockerels given *Vernonia amygdalina* leaf extract in varying concentration. The observation showed that all biochemical parameters evaluated were not significantly influenced by the dietary treatment except that of triglyceride. Triglyceride values of birds which received 0.4mg/l Vit C (T2) was significantly ($P<0.05$) lower, while the values recorded for birds in T3, T4 and T5 (20.06, 20.19 and 22.06) respectively, were not significantly different from the control value. Although not significantly different across the treatments, the highest values of total cholesterol (277.08mg/dl) and low density lipoprotein (248.62mg/dl) were recorded in T1 while the least values (88.54mg/dl) and (74.30mg/dl) respectively were recorded in Treatment 2. Cholesterol and triglycerides is important constituent of the lipid fraction of animal body. Cholesterol is essential for the normal functioning of all animal cells and a fundamental element of cell membranes. Also they act as a precursor for some hormones, when present in high amount above the upper limit of the physiological normal range (52-148mg/dl) in chickens, it will pose a threat to the health of the birds. However, Aspartate amino transferase (AST) shows a significant different among the treatment fed varying VALE. This is possibly due to the increase of liver metabolism and to the significant muscle development that usually happens during this period, as observed by Szabo *et al.* (2005) in turkeys of commercial strains. AST is a liver enzyme in which high amount of it indicates liver infection or damage. A high value of AST is recorded in T4 (78.87U/L) which receives 60mls of VALE and T2 (73.80U/L) which receives 0.4mg of Vitamin C/ litre. Birds under T5 (74.00) that receives 90mls of the VALE had values which was also significantly lower than the control. The result of their study showed that supplementation of 90ml of concentration of *Vernonia* extract made available sufficient minerals for normal functioning of the liver, cells, bone and osteocytes due to signifance difference found in AST and Triglycerides.

CONCLUSION

In conclusion, the result of this showed that Vernonia amygdalina leave have are nutritional requirements of poultry. Perhaps the leave extract of this plant could be used as feed supplement and as medicine in poultry to improve health status. It is concluded that blood profile and serum biochemistry of cockerels fell within reported values. Supplementation of *Vernonia amygdalina* leaves extract via drinking water showed a moderate effect on overall haematology and serumbiochemistry of heat stressed cockerels. However, supplementation of 90mls of VALE in poultry especially cockerels will not adversely affect the blood profile and the health status of the animals.

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